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DEVELOPMENT OF A METHOD TO DETERMINE THE AUDIOGRAM OF THE GUINEA PIG FOR THRESHOLD SHIFT STUDIES

By Carlos Comperatore James H. Patterson, Jr.

SENSORY RESEARCH DIVISION

January 1984

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U.S. ARMY AEROMEDICAL RESEARCH LABORATORY FORT RUCKER, ALABAMA 36362

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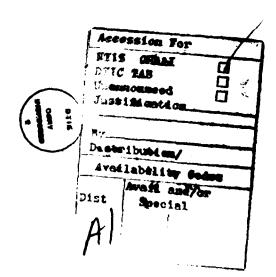
ABSTRACT: Studies of noise induced threshold shift in guinea pigs require a method to determine the audiogram which meets the following criteria: It must be reliable and permit the determination of threshold at eight to twelve frequencies in a single session lasting less than one hour. A conditioned suppression procedure was adopted to meet these requirements. Three guinea pigs were trained and a series of audiograms determined on each. The audiograms were found to be reliable and in good agreement with published audiograms determined by other methods.

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TABLE OF CONTENTS

																							PAGE NO.
List of Tables	s		•		•		•		•	•	•	•	•	•	•	•	•	•		•	•	•	1
List of Figure	es		•	•			•		•	•	•	•	•	•		•	•				•	•	2
Introduction .				•	•		•	•	•	•	•	•	•	•			•						3
Methods and Ma	ateria	als.				•	•	•	•	•	•	•	•	•	•	•	•	•				•	4
Results and Di	iscus	sion	•	•	•	•	•	•		•	•		•	•		•	•	•	•	•	•	•	7
Conclusions						•	•	•	•	•	•	•		•	•	•	•		•	•	•	•	15
References				•	•	•	•		•	•	•	•		•					•		•	•	17
Appendix A. l	List	of Ed	iup	þπ	en	t	Ма	ını	ıfa	ıct	uı	rei	·s	•							•		19



LIST OF TABLES

TABLE	NO.	PAGE NO.
1	Means and Standard Deviations of the Last Five Audiograms Determined During Audiometric Training of Three Guinea Pigs	13
	LIST OF FIGURES	
FIGURE	NO.	PAGE NO.
1	Average threshold in dB (SPL) as a function of audiometric training session for four guinea pigs	9
2	Average audiogram for subjects G2, 7C, and 7D determined during training sessions 10 through 14. Vertical bars indicate one standard deviation across subjects	10
3	Average audiogram for subjects G2, 7C, and 7D determined during training sessions 20 through 24. Vertical bars indicate one standard deviation across subjects	11
4	Average audiogram for subjects G2, 7C, and 7D determined during last five training sessions. Vertical bars indicate one standard deviation across subjects	14

INTRODUCTION

The present study was undertaken to establish a method for performing behavioral audiometry on the guinea pig suitable for use in a noise-induced threshold shift experiment. The basic paradigm utilized to determine threshold shift consists of establishing a baseline audiogram before noise exposure and then determining one or more audiograms thereafter. This paradigm places certain constraints on the audiometric procedure which can be used. First, it requires reliability since a succession of audiograms are used to trace the effects of the exposure. Second, the entire audiogram consisting of eight to twelve frequencies must be determined in a single session. Third, the test session should be less than I hour in order to permit the detection of effects which are present immediately after the exposure and rapidly disappear. Finally, the reinforcement and motivation used during behavioral training should remain as constant as possible to avoid contaminating the time-varying effects of noise exposure on hearing sensitivity.

Various behavioral methods have been used to measure the guinea pig's auditory threshold. Shock avoidance is a procedure commonly used with other species. In an early attempt to study escape responses present within the guinea pig's behavioral repertoire, Anderson and Wedenberg (1965) signaled electric shock with a tone in an avoidance paradigm. Their results showed that their subjects did not learn to avoid shock even after 3.000 training trials. This finding was attributed to the guinea pig's tendency to become immobile in response to aversive stimulation. Later data on the guinea pig's immobility response (Miller and Murray, 1966) confirmed that shock avoidance training would not result in a consistent response in the presence of auditory stimuli. Capitalizing on the immobility response to aversive stimuli, Anderson and Wedenberg developed a method based on the suppression of an ongoing behavior. In this case, the ongoing behavior consisted of the induction of shivering as a result of being maintained in low environmental temperature. When audible tones, previously paired with shock, were presented, the shivering behavior was suppressed. This paradigm is an adaptation of the conditioned suppression phenomenon previously described by Estes and Skinner (1941). Anderson and Wedenberg exposed guinea pigs to pure tones of 2000 Hz at 60 dB sound pressure level (SPL), with each being followed by a brief electric shock. This training was carried out for six sessions. Shivering then was induced by blowing cold air over the subjects. Audiograms were determined by recording shivering interruptions upon tone presentations. According to Anderson and Wedenberg, reliability of audiograms depended upon maintaining the duration of intertrial intervals at no more than 30 seconds, and actual sessions at 45 minutes or less. In addition, cooling had to be carefully regulated in order to maintain a stable rate of shivering.

In a more recent effort, Crifo (1973) refined the method used by Anderson and Wedenberg by improving the training procedure as well as the testing apparatus. Crifo named this improved method shiver audiometry.

While Crifo's method appears to produce reliable audiograms, methodological difficulties in maintaining low environmental temperatures (0° to 2° Celsius) and in recording the shivering response make this procedure somewhat cumbersome to implement in a noise exposure paradigm.

Miller and Murray (1966) also used a method of suppressing ongoing behavior. They measured guinea pigs' auditory thresholds by presenting pure tones superimposed on ongoing lettuce chewing. Each presentation inhibited chewing, but also resulted in gradual stimulus habituation. Testing ten frequencies in a single session of approximately 60 minutes duration was not considered possible with this method. In addition, the noise of chewing could elevate the measured threshold.

More recently, Prosen et al., (1978) determined threshold for a frequency range between 125 Hz and 52 kHz by using a positive reinforcement training method. In this procedure, tones served as discriminative stimuli for a report response. Guinea pigs first were trained to depress a key with their noses. The key was located to the left of a food magazine. Tone presentations were used as discriminative stimuli to depress a second key located to the right of the food magazine. This response was reinforced and the trial ended. This method yielded more sensitive thresholds below 4.0 kHz than previously published audiograms for guinea pigs (Miller and Murray, 1966; Heffner et al., 1971). Above 4.0 kHz thresholds obtained were comparable to previous data. By this method, Prosen et al., were able to test five to six frequencies per day. While this method appears to produce lower thresholds than previously reported methods, the inability to determine a complete audiogram in a single session suggested that it would not be suitable for noise-induced threshold shift studies.

Also using guinea pigs, Heffner et al., (1971) developed a variation of the conditioned suppression paradigm. Water deprived animals operantly were conditioned to lick a drinking tube for water reinforcement. Tone-shock pairings then were superimposed on this previously trained-licking response. After tone-shock presentations, subjects reduced their licking rate whenever an audible tone was presented. Changes in licking rate as a function of intensity were used to determine thresholds. Since this method provides for stable ongoing behavior, the animals' motivation is under control of the deprivation schedule and the responses are relatively easy to quantify. While none of these procedures yielded complete audiograms in single sessions, the method by Heffner et al., appeared to be most adaptable for threshold shift testing. In this report, the initial audiogram testing method was derived from the basic conditioned suppression response paradigm described by Heffner et al.

METHODS AND MATERIALS

Three albino and three pigmented male guinea pigs, <u>Cavia Parcellus L.</u>, of the English variety were obtained from the US Army Aeromedical Research Laboratory (USAARL) breeding colony to serve as subjects for this study.

Albino guinea pigs G1, G2, and G3 were 7 months old when they entered the study. The three pigmented guinea pigs 7C, 7D, and 7B, were 3 months old at the beginning of the training.

Testing and training sessions were carried out in a sound-treated chamber consisting of a room within-a-room, manufactured by Industrial Acoustics Company.* During testing, subjects were placed in a 20- by 20-cm grid floor cage. An 8- by 8-cm plastic board was attached to the front wall. An orifice in this board allowed the tip of a 7-mm drinking tube to protrude into the experimental cage. Water was supplied to the spout by means of water pressure exerted via a 5-gallon plastic container located 2.5 meters above the floor outside the experimental chamber. Water reinforcement was dispensed by means of an electronic solenoid valve, which in turn was controlled by previously programmed Coulbourn* logic circuit modules. Licking responses were monitored by a Coulbourn counter which was triggered each time the subjects' tongue touched the drinking tube.

During tone presentations, selected frequencies were generated by a Fluke Signal Generator,* model number 6010A. Its output then was driven through a Coulbourn Selectable Envelope-Shaped Rise/Fall Gate, model number 584-04, set for exponential rise/fall. The output of the gate was connected through a Hewlett-Packard* model 350D attenuator to an Altec* model 1954B power amplifier. The output of the amplifier was connected through a Grason-Stadler* model 1293 10 ohm attenuator to an Altec model 604-8H coaxial speaker in a model 612C cabinet. Tones were presented in the experimental chamber via the speaker located 1.1 m from the experimental cage. In addition, an Altec model T0249 monitor speaker was used to monitor each stimulus presentation.

Subjects' activity within the experimental environment was visually monitored via a video camera located inside the chamber. Outside the chamber, a television receiver/monitor was used to display the camera's output.

During training and testing trials, footshocks were administered by means of a Coulbourn shock scrambler, model number E-13-16. In addition, a buzzer, driven through a 1-inch tweeter attached to the side of the cage, was presented at the offset of the shock and replaced the shock when selected by the experimenter. Finally, all stimulus presentations, as well as schedules of reinforcement and suppression ratio calculations, were automatically controlled via a 6800 CPU microprocessor interfaced to the Coulbourn logic modules.

The sound field was calibrated using a Bruel and Kjaer* 1/2-inch condenser microphone; a Bruel and Kjaer-type 2804 battery-powered microphone power supply; a Bruel and Kjaer-type 2606 measuring amplifier and a Federal Scientific* model 440A spectrum analyzer. Measurements of the sound pressure level (SPL) at three locations for each test frequency were made. These locations were chosen to approximate the range of animal head positions which might

^{*}See Appendix A.

occur during licking. The average level at each frequency across all measurement locations was used as the final calibration value.

Initially, the six experimental subjects were exposed to the test environment after 24 hours of complete water deprivation in their home cages. Subjects were placed in the experimental cage and allowed to spontaneously discover the location of the drinking tube. Throughout the first 10 days of training, the licking tube, initially protruding into the experimental cage, gradually was pulled back out of the cage so that it could barely be reached by the subjects' tongue. This procedure prevented animals from biting the drinking tube, and gradually contributed to stabilization of the ongoing licking rate. In addition, another contributing factor to the development of a continuous, uninterrupted, stable licking rate consisted of the implementation of the proper water deprivation schedule. During training days, subjects were allowed to obtain water only from the experimental drinking tube at a fixed ratio (FR) l rate. On the nontraining days subjects remained in their home cages and received 30 ml of tap water daily. Consequently, subjects gradually experienced a weight loss from 10% to 23% of their original predeprivation body weight. This deprivation procedure proved adequate throughout training for both the subjects' proper physiological health and the maintenance of the necessary level of motivation to establish a stable licking rate in the course of a 60-minute session. Over the course of the training it was possible to raise the FR schedule of reinforcement to 10 licking responses per reinforcement (FR10).

Audiogram tests were initiated upon the fourth week of training and continued throughout the experiment. For each audiogram, 10 frequencies were tested: 125 Hz, 250 Hz, 500 Hz, 1.0 kHz, 1.4 kHz, 2.0 kHz, 2.8 kHz, 4.0 kHz, 5.7 kHz, and 8.0 kHz. Each session consisted of 100 trials, 10 trials at each of the 10 frequencies. Each trial consisted of a base period of 4.5 seconds during which the subjects' operant licking rate was determined. This was followed by a stimulus presentation interval of 4.5 seconds during which the subjects' licking rate was determined. Each stimulus consisted of a pulsed tone. Three pulses of 750 msec on and 750 msec off per pulse. The stimulus interval was followed by the reinforcement interval, during which an aversive stimulus under the control of the experimenter could be applied. The aversive stimulus, a .7 ma shock, was introduced in 2 of the 10 signal levels tested at each frequency. Footshocks followed tones at high and low levels, so that tones at all levels would become conditioned to shock. As conditioning proceeded, tones always were presented superimposed upon the ongoing licking response. A cessation or reduction of licking rate during the stimulus interval indicated a tone was audible. Each frequency was tested at 10 levels varying in 10 dB steps over a 90 dB range.

On each trial, suppression was quantified by counting the number of licking responses emitted during the base period (P) and the number of responses during the stimulus presentation (W). The suppression ratio then was computed: SR = W/P+W. When a suppression is complete, W becomes zero and the suppression ratio becomes zero. When there is no suppression, W is

approximately equal to P and the suppression ratio will be approximately .5. In order to categorize a trial as having shown suppression or not, a criterion value of .25 was adopted. Suppression ratios at or below .25 were interpreted as an indication of active licking suppression in response to signal detection, while values above .25 indicated no suppression, thus poor or no signal detection. The threshold for a test frequency was calculated by linear interpolation between the lowest signal level which yielded the suppression ratio above .25 and the next lower signal level. After a complete audiogram was determined, an index was calculated by averaging the threshold sound pressure levels across frequencies. This average was used as an overall performance measure to assess the progress of training.

RESULTS AND DISCUSSION

During the development of the training method, the deprivation duration and the number of sucks presented during a given session were identified as variables which had to be maintained within specific boundaries. During the first few sessions on which test trials were presented, subjects produced long periods of immobility, apparently in response to the test tones. This response was so persistent that only one or two trials could be presented in the 1 hour allotted to each subject instead of the 100 scheduled. In order to habituate this reaction to the test tones, shock was suspended and tone trials were presented independent of the subjects' licking behavior. After approximately 20 sessions, the immobility response had diminished to a point where shock could be resumed. Then it was decided to administer shocks only in 10% of all trials per session and on one of the 10 levels of each frequency in a random fashion. After several sessions, subjects began to lick through stimulus intervals at tone levels well above threshold. The proportion of shock trials then was gradually increased in an attempt to bring licking suppression under better stimulus control. When the shock density became too high (above 50%), subjects' licking responses became irregular and suppression ratios became unreliable. Therefore, shock densities were maintained between 10 to 20% for the remainder of the experiment.

On the other hand, the water deprivation schedule directly influenced subjects' motivation to develop consistent licking rates. It was noted that 28 hours or more of water deprivation caused subjects to lick at unusually high rates, to rapidly satiate and to cease licking prior to the completion of the full set of test frequencies. Consequently, water deprivation was limited to less than 24 hours.

These early sessions can be considered as pretraining since the data were so erratic that no estimate of the audiograms could be derived. This pretraining phase lasted 54 sessions. During pretraining two subjects, G1 and 7B, were dropped from the study for failing to make progress toward a complete audiogram.

The next 52-55 sessions yielded an estimate of a complete audiogram for subjects 7D, 7C, G2, and G3 on most of the sessions. These sessions we call

audiometric training. To quantify any improvement in the estimated thresholds over training sessions, the "threshold" sound pressure level was averaged across frequencies for each session to produce an average threshold. Figure 1 shows this average threshold as a function of audiometric training session. Subject G3 was dropped from the study after 24 sessions due to inconsistent patterns of responding and increasing numbers of incomplete audiograms.

At first, early threshold estimates were very high. Over the first 14 sessions audiograms improved to an apparent asymptote. The audiograms obtained on training sessions 10 to 14 were averaged for subjects 7D, 7C, and G2 and then means and standard deviations for the group of subjects were computed. Figure 2 shows this average audiogram. For reference, the audiograms reported by Heffner et al., and Prosen et al., are shown in Figure 2. Our audiogram appears to be elevated and shows considerable variability between subjects (large standard deviations).

These results suggested the possibility that our subjects may not have been suppressing licking as a function of conditioned aversiveness of the tones. Close behavioral observations made during training corroborated this suspicion. Subjects were found to behaviorally respond to the onset of a stimulus interval by orienting towards the stimulus source, but resumed licking almost immediately in the case of low intensity stimuli. Consequently, it was possible to infer that the aversive conditioning method used did not produce the necessary conditioning required to suppress the licking behavior long enough to reduce the suppression ratio below .25.

Analysis of the initial training method suggested that the pulsed tones presented during the stimulus interval may have contributed to this apparent lack of conditioning. If each tone pulse (three per stimulus interval) was a separate discriminable event, the occurrence of the shock at the end of three tones on only 10% of the trials resulted in an actual tone-shock pairing on less than 4% of the tones and never on the first or second tone of the trial. This may have been an inadequate shock density to support aversive conditioning of the tones.

Thus, in order to increase the reliability of the tone as a source of information for shock presentations, two modifications of the training method were implemented. Our first step was to replace the pulsed tone stimulus by a single 4.5-s tone. This was done on the fifteenth training session. Then, for approximately 10 sessions, subjects were trained under the same schedule of aversive stimulation as in earlier sessions. This step was a precautionary measure to prevent possible detrimental effects resulting from changing more than one stimulus parameter at a time.

To determine the effect of changing from pulsed to 4.5-s steady tones, an average audiogram was computed on training sessions 20 to 24. These results are shown in Figure 3. There was a slight improvement in the average audiogram and a reduction in the standard deviations. This was primarily due to an improvement in the audiogram of G2 (see Figure 1). However, the group audiogram still is elevated compared to the published

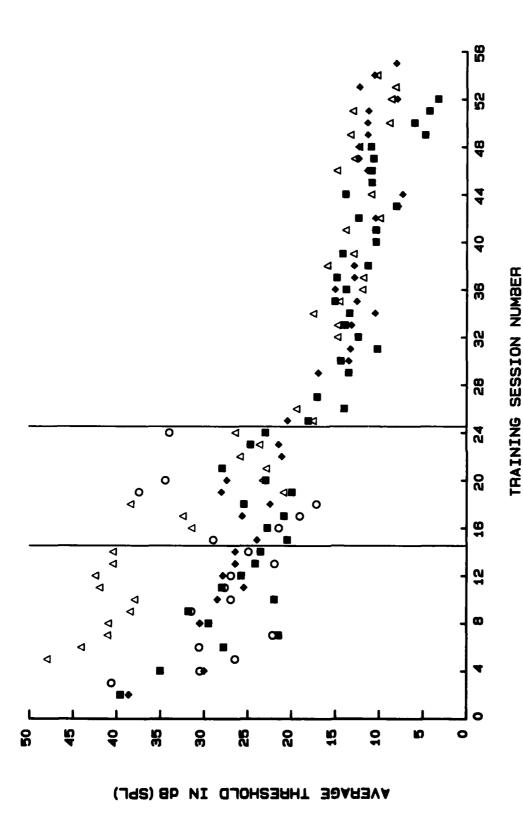
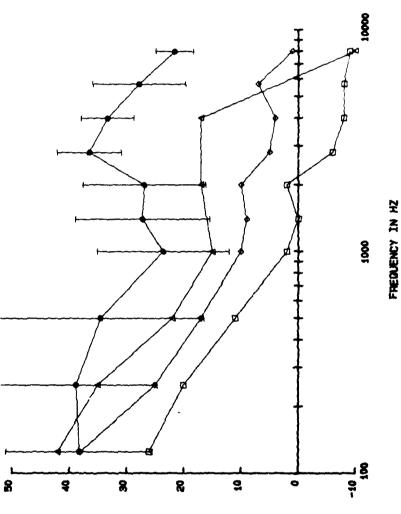
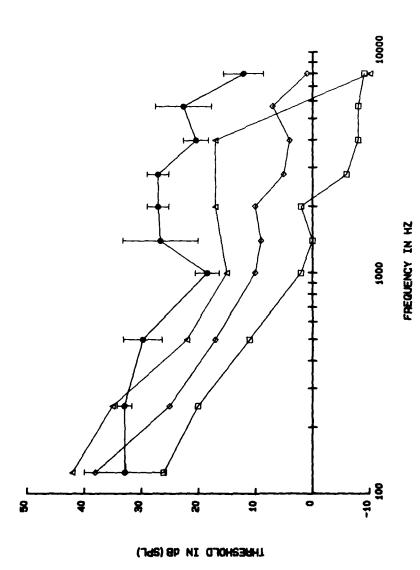


FIGURE 1. Average threshold in dB (SPL) as a function of audiometric training session for four guinea pigs: \spadesuit , %; \blacksquare , %; \triangle , %; \triangle , %?.



THRESHOLD IN AB (SPL.)

Average audiogram for subjects G2, 7C, and 7D determined during training sessions 10 through 14. Closed symbols: •, present study; Open symbols: △, Heffner et al., (1971); ◇, Prosen, et al., (1978), Albino; □, Prosen et al., (1978) Pigmented. Vertical bars indicate one standard deviation across subjects. FIGURE 2.



Average audiogram for subjects G2, 7C, and 7D determined during training sessions 20 through 24. Closed symbols: ●, present study; Open symbols: △, Heffner et al., (1971); ◇, Prosen, et al., (1978), Albino; □, Prosen et al., (1978) Pigmented. Vertical bars indicate one standard deviation across subjects. FIGURE 3.

results.

At the 25th session, the percentage of tone-shock pairings was increased to 100% by introducing a classical conditioning session prior to the actual audiogram test. Each preaudiogram session consisted of 40 trials. This permitted tone-shock pairing on four intensity levels at each of the ten frequencies. The tones were presented at sound pressure levels equal to and below previously published threshold data by Heffner et al. and Prosen et al.

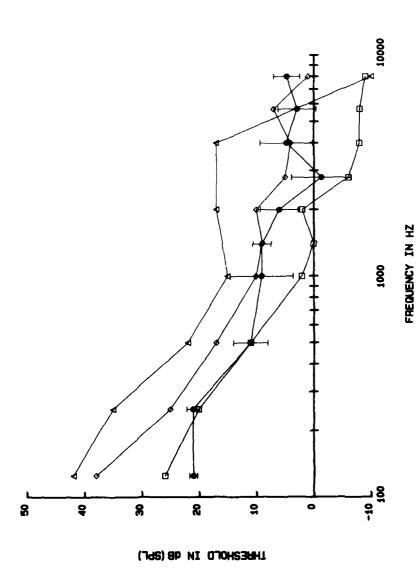
At the beginning of each classical conditioning session, the drinking tube was hidden in order to prevent the development of any possible contingency between the licking device and shock. Subjects then were exposed to tone-shock pairings until all 40 trials were presented. After a short time-out of approximately 5 minutes, the drinking tube was reintroduced in the experimental environment, thus marking the beginning of the audiogram session. Throughout the testing session, tones were presented at SPL values comparable to those used in the classical conditioning sessions. Footshocks also appeared in levels previously shocked during classical conditioning sessions, but on approximately 50% of all trials. Extinction effects of the testing session were minimized in this manner. In addition, footshocks also were administered contingent on licking during the presentation of a tone. When subjects maintained the licking response throughout a trial, a shock was presented near the offset of the stimulus interval. Then the level tested was considered a training trial and the same intensity level tested once more. If similar results were obtained, the above procedure was repeated for a last time. These results then were recorded and the next two 5 dB steps tested. This procedure provided our testing method with the means to drive our subjects' suppression responses to the lowest possible SPL values.

Examination of Figure 1 shows the improvement in average threshold during sessions 25 to 56 which resulted from this last procedural modification. These results point to a potential methodological pitfall. The occurrence of an apparent asymptote in the average audiogram from the 15th to 25th sessions (Figure 1) could lead to the incorrect conclusion that this is the best audiogram for each subject. Indeed, if the data of Figure 3 are corrected for pinna effects it would be in general agreement with the audiograms obtained by Heffner et al., except at 8000 Hz. However, it is clear that the guinea pig audiogram is significantly better than indicated by this early plateau. The primary methodological difference between the early asymptote and the later one is that the reinforcement contingencies were modified to motivate the subject to produce his "best" audiogram during later sessions. Table I contains the means and standard deviations for each subject over the last five audiograms. The standard deviations generally are small, indicating that by this stage in training each subject is producing a reliable audiogram. Figure 4 shows the average audiogram for the group based on these last five training sessions. Note that below 1000 Hz our audiogram is in good agreement with the better of the two groups from Prosen et al. At higher frequencies, our audiogram shows higher threshold values

TABLE 1

MEANS AND STANDARD DEVIATIONS OF THE LAST FIVE AUDIOGRAMS DETERMINED DURING AUDIOMETRIC TRAINING OF THREE GUINEA PIGS

						Frequ	Frequency					
Subject		125 Hz	250 Hz	500 Hz	1.0 kHz		1.4 kHz 2.0 kHz	2.8 kHz	4.0 kHz	5.7 KHz	8.0 kHz	
29	Mean	21.1	21.8	17.4	14.9	16.2	11.2	3.1	12.9	6.7	5.2	
	S.D.	5.5	4. €.	2.2	2.7	2.2	2.7	3.5	4.2	2.7	5.5	
20	Mean	23.2	(U)	16.0	14.9	13.2	10.2	6.1	6.1	3.7	4.2	•
	s. ₅ .	2.7	т. т.	2.7	2.7	2.2	2.7	2.7	2.7	7.1	5.7	
02	Mean	22.2	20.8	16.4	10.9	14.2	6.2	2.1	9.9	6.7	9.9	
	S.9.	2.7	2.7	2.7	4.2	4.5	2.7	8.2	4.7	2.7	4.6	



Average audiogram for subjects G2, 7C, and 7D determined during last five training sessions. Closed symbols: ●, present study; Open symbols: △, Heffner et al., (1971); ◇, Prosen, et al., (1978), Albino; □, Prosen et al., (1978) Pigmented. Vertical bars indicate one standard deviation across subjects. FIGURE 4.

than their better group.

The elevated thresholds at higher frequencies probably are a result of the arrangement of the sound source and the test cage. The speaker was located at approximately 1350 to the right of the subject while he was licking, i.e., to the side and behind the subject. This orientation would provide a "shadowing" of the higher frequencies by the pinna. The pinna effect is estimated to be on the order of 5 dB (Sinyor and Laszlo, 1973). With this correction, our audiogram would approach the better audiogram reported by Prosen et al.

CONCLUSIONS

Complete 10-frequency audiograms were obtained in less than 1 hour. The audiograms produced by this conditioned suppression method are in good agreement with published audiograms for guinea pigs. The audiometric data produced by this method appear to be stable over weeks, permitting the determination of recovery from threshold shift functions.

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